Non-Invasive Prenatal Test for beta-Thalassemia and Sickle Cell Disease Using Probe Capture Enrichment and Next-Generation Sequencing of DNA in Maternal Plasma.

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Public Summary:
BACKGROUND: Noninvasive prenatal testing (NIPT) of chromosomal aneuploidies based on next-generation sequencing (NGS) analysis of fetal DNA in maternal plasma is well established, but testing for autosomal recessive disorders remains challenging. NGS libraries prepared by probe capture facilitate the analysis of the short DNA fragments plasma. This system has been applied to the beta-hemoglobinopathies to reduce the risk to the fetus. METHOD: Our probe panel captures >4 kb of the HBB region and 435 single-nucleotide polymorphisms (SNPs) used to estimate fetal fraction. Contrived mixtures of DNA samples, plasma, and whole blood samples from 7 pregnant women with beta-thalassemia or sickle cell anemia mutations and samples from the father, sibling, and baby or chorionic villus were analyzed. The fetal genotypes, including point mutations and deletions, were inferred by comparing the observed and expected plasma sequence read ratios, based on fetal fraction, at the mutation site and linked SNPs. Accuracy was increased by removing PCR duplicates and by in silico size selection of plasma sequence reads. A probability was assigned to each of the potential fetal genotypes using a statistical model for the experimental variation, and thresholds were established for assigning clinical status. RESULTS: Using in silico size selection of plasma sequence files, the predicted clinical fetal genotype assignments were correct in 9 of 10 plasma libraries with maternal point mutations, with 1 inconclusive result. For 2 additional plasmas with deletions, the most probable fetal genotype was correct. The beta-globin haplotype determined from linked SNPs, when available, was used to infer the fetal genotype at the mutation site. CONCLUSION: This probe capture NGS assay demonstrates the potential of NIPT for beta-hemoglobinopathies.

Scientific Abstract:
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